See related Commentary on page xvi

Prevention of Murine Erythropoietic Protoporphyria-Associated Skin Photosensitivity and Liver Disease by Dermal and Hepatic Ferrochelatase

Robert Pawliuk,* Robert Tighe,* Robert J. Wise,† Micheline M. Mathews-Roth,‡ and Philippe Leboulch*§

*Massachusetts Institute of Technology, Division of Health Sciences & Technology, Cambridge, Massachusetts, USA; †Harvard Medical School and Hematology Division, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA; ‡Harvard Medical School and Channing Laboratory, Department of Medicine, Brigham & Women's Hospital, Boston, Massachusetts, USA; §Harvard Medical School and Division of Genetics, Department of Medicine, Brigham & Women's Hospital, Harvard New Research Building, Boston, Massachusetts, USA

Erythropoietic protoporphyria (EPP) is caused by a defect in ferrochelatase, leading to the accumulation of protoporphyrin predominantly in erythrocytes and hepatocytes, and resulting in skin photosensitivity upon leaching of blood protoporphyrin into the skin. Some patients also develop severe liver damage. Because the respective contributions of hepatic and erythrocytic protoporphyrin to the pathophysiology of EPP remain unclear, we investigated this question using the murine model of EPP. Transplantation of bone marrow from EPP mice to normal recipients resulted in elevated erythrocyte and plasma protoporphyrin levels. However, quantification of serum liver enzymes and bilirubin together with histopathologic examination of liver sections of mice up to 16 months post-transplantation showed no evidence of liver damage. Moreover, despite massive elevation of serum protoporphyrin, transplanted mice showed minimal evidence of skin photosensitivity. Photosensitivity could also be prevented locally by implanting skin grafts from normal mice onto the backs of EPP recipients. These data validate the hypothesis that the main source of toxic protoporphyrin originates from the erythrocytes. However, we unexpectedly observed that normal ferrochelatase activity in hepatic and dermal cells of wild-type mice is sufficient to prevent liver disease and significant skin photosensitivity. These findings may provide new strategies for the treatment of EPP.

Key words: cirrhosis/erythropoietic protoporphyia/ferrochelatase/photosensitivity
J Invest Dermatol 124:256–262, 2005

Erythropoietic protoporphyria (EPP) is a hereditary disorder of porphyrin metabolism in which the activity of ferrochelatase, the enzyme responsible for inserting iron into protoporphyrin to form heme, is reduced (Desnick, 1994). Although ferrochelatase is produced in all tissues of the body, this decrease in ferrochelatase activity (15%–40% of normal) leads to the accumulation of large amounts of protoporphyrin in erythrocytes because of the fact that porphyrin metabolism is especially active in these cells (Desnick, 1994). The accumulated protoporphyrin leaks into the plasma, ultimately producing the clinical symptom that is the hallmark of this disease: severe photosensitivity of light-exposed skin resulting in itching, burning, and rarely ulceration (Desnick, 1994). Moreover, approximately 5% of EPP patients are at risk for developing cholestasis from the abnormal accumulation of excess protoporphyrin in hepatocytes, causing progressive liver damage and eventual liver failure (Bloomer, 1999). Other rare systemic symptoms include hemolytic anemia and peripheral neuropathy (Key *et al*, 1992).

Abbreviations: EPP, erythropoietic protoporphyria; HSC, hematopoietic stem cell

Currently, treatment for EPP consists of preventing or lessening photosensitization by ingesting β-carotene. This treatment is, however, ineffective in approximately 20% of patients (Mathews-Roth *et al*, 1977) and does not protect against liver damage. Alternatively, bone marrow transplantation has the potential to reduce or eliminate erythrocytic protoporphyrin (Poh-Fitzpatrick *et al*, 2002), although the feasibility of this approach is limited by the requirement for a human leukocyte antigen-matched donor. Autologous transplantation of genetically modified bone marrow remains an attractive alternative in the absence of a suitable donor. Bone marrow transplantation with either allogeneic cells or autologous, genetically modified cells would, however, not protect the patient from cirrhosis and eventual liver failure, if hepatic-derived protoporphyrin plays a significant role in the development of liver damage. The role of hepatic protoporphyrin production in the development of liver damage remains unclear (Bloomer, 1999). A detailed understanding of how hepatic and erythrocytic-derived protoporphyrin contribute to photosensitivity and liver disease is critical to the development of strategies aimed at treating EPP.

A mouse model of EPP, BALB/C-Fech^{m1Pas}, is characterized by a profound deficiency in ferrochelatase enzyme activity (less than 6% of normal control mice) caused by a

missense mutation in the ferrochelatase gene (Tutois *et al*, 1991; Boulechfar *et al*, 1993). These mice demonstrate high levels of protoporphyrin in erythrocytes, plasma, liver, and stool and, importantly, show all the clinical features observed in human EPP patients including hemolytic anemia, photosensitivity, cholestasis, and severe liver dysfunction (Tutois *et al*, 1991; Boulechfar *et al*, 1993). In contrast to human patients, all BALB/C-Fech^{m1Pas} mice develop irreversible liver damage (Tutois *et al*, 1991). We have previously utilized this mouse model to demonstrate long-term cure of photosensitivity by retrovirus-mediated, preselective hematopoietic stem cell (HSC) gene therapy (Pawliuk *et al*, 1999). More recently, other groups have confirmed our findings and demonstrated either complete or partial correction of photosensitivity in EPP mice with or without preselection of transduced cells (Fontanellas *et al*, 2001; Richard *et al*, 2001).

Using BALB/C-Fech^{m1Pas} mice as a model of human EPP, we sought to define, more precisely, the relative contributions of hepatic and erythrocytic protoporphyrin to the development of EPP-associated photosensitivity and liver disease. Transplantation of bone marrow from BALB/C-Fech^{m1Pas} mice into congenic BALB/C recipients confirmed that the vast majority of protoporphyrin derives from the red blood cell compartment and demonstrated that in the presence of normal cellular levels of hepatic and dermal ferrochelatase, elevated plasma protoporphyrin levels alone are insufficient to generate EPP-associated liver disease and significant photosensitivity.

Results

Elevation of erythrocytic protoporphyrin concentration and the percentage of protoporphyrin fluorescent red blood cells in normal BALB/C recipients of EPP donor marrow Femoral bone marrow was isolated from female EPP donors and 7.2×10^6 cells were injected intravenously into irradiated male BALB/C recipient mice. As a control, bone marrow was also harvested from normal female BALB/C donors and 8.3×10^6 cells were transplanted into irradiated male BALB/C recipients. A total of five and four recipients were transplanted with EPP and BALB/C donor marrow, respectively. Mice were kept for long-term studies and monitored at regular intervals by biochemical and fluorescent activated cell sorting (FACS) analysis. At 3 mo post-transplantation, intracellular protoporphyrin concentrations were greatly elevated in the red blood cells of BALB/C mice transplanted with EPP marrow and were indistinguishable from the non-transplanted EPP control mouse (Fig 1*A*). As expected, the concentration of protoporphyrin in red blood cells from BALB/C mice transplanted with normal BALB/C marrow was not elevated and was essentially identical to a non-transplanted BALB/C control mouse (Fig 1*A*). These levels remained relatively constant for at least 16 mo post-transplantation, the longest time point assessed.

Next, to investigate the reconstitution of the erythroid compartment with red blood cells derived from the abnormal EPP donor bone marrow, we analyzed circulating red blood cells from recipient mice by FACS. Protoporphyrin

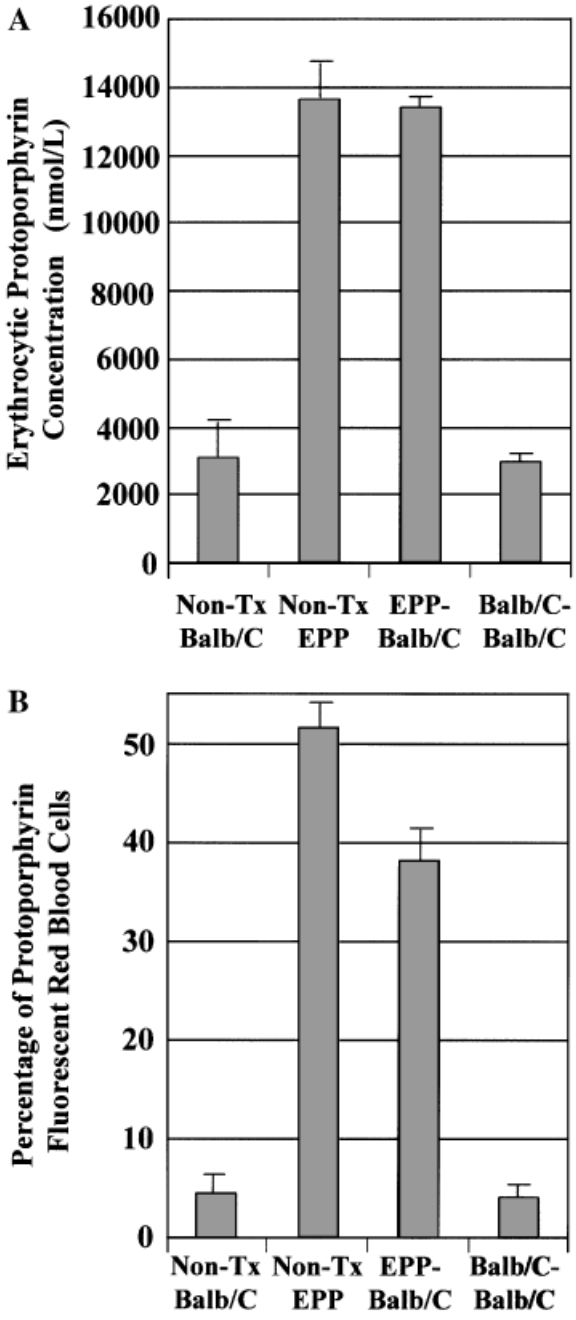

Figure 1
Concentration of erythrocytic protoporphyrin (*A*) and percentage of protoporphyrin fluorescent red blood cells (*B*) 3 mo post-transplantation. BALB/C recipient mice were transplanted with either disease erythropoietic protoporphyria (EPP) marrow (EPP-BALB/C) (five mice) or normal marrow (BALB/C–BALB/C) (three mice). Two non-transplanted (Non-Tx) BALB/C and two EPP mice were used as controls.

was excited at 488 nm whereas a 650 nm filter was used to detect protoporphyrin-specific fluorescence at 3 mo post-transplantation. Flow cytometric analysis of circulating red blood cells from normal BALB/C recipients transplanted with EPP marrow showed that the proportion of protoporphyrin fluorescent red blood cells was comparable with that observed in unmanipulated EPP disease mice ($38\% \pm 2.2\%$ (standard deviation (SD)) *vs* $51.5\% \pm 3.3\%$ (SD); $p > 0.1$) and greatly elevated as compared with

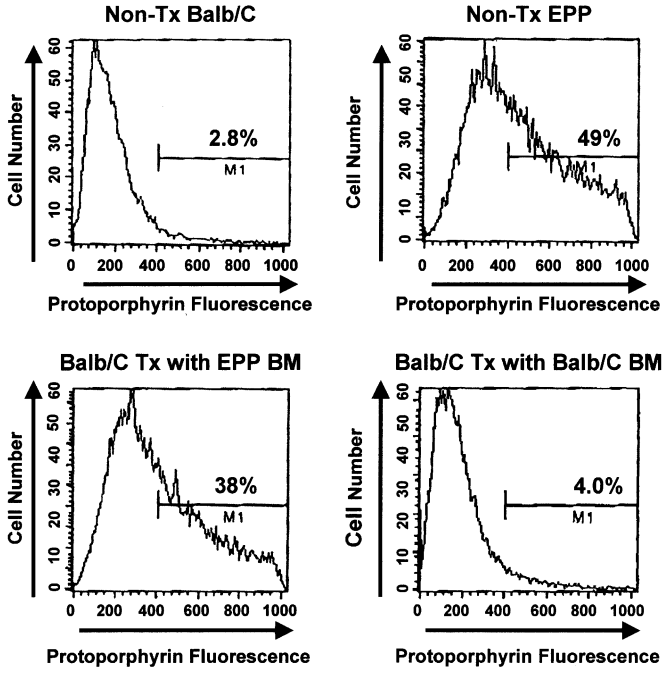

Figure 2
Fluorescent activated cell sorting analysis of red blood cells for protoporphyrin specific fluorescence 3 mo post-transplantation. Non-transplanted (Non-Tx) BALB/C and erythropoietic protoporphyria (EPP) mice were used as controls. The percentage of protoporphyrin fluorescent red blood cells is shown. BM, bone marrow.

recipients of normal BALB/C donor marrow (38% $\pm$ 2.2% (SD) *vs* 3.9% $\pm$ 1.6% (SD); $p < 0.001$) (Figs 1*B* and 2).

These data demonstrate high level engraftment of BALB/C recipient mice with transplanted donor EPP bone marrow leading to the production of circulating red blood cells with a greatly elevated intracellular and plasma protoporphyrin levels.

Absence of liver damage in normal BALB/C mice transplanted with EPP donor marrow EPP model mice are characterized by severe hepatic dysfunction including biliary cirrhosis, regenerative hyperplasia, extracellular deposition of protoporphyrin, and a marked elevation in serum concentrations of bilirubin, alkaline phosphatase, aspartate amino-transaminase (AST), and alanine amino-transferase (ALT) (Boulechfar *et al*, 1993). To investigate whether the elevated levels of protoporphyrin in the plasma of normal BALB/C recipients of EPP disease marrow was sufficient to generate liver damage, serum levels of bilirubin, alkaline phosphatase, AST, and ALT were monitored at regular intervals. As shown in Table I, the concentration of liver enzymes and bilirubin in serum collected from BALB/C recipients transplanted with EPP marrow were comparable with non-transplanted control BALB/C mice and BALB/C mice transplanted with normal BALB/C marrow. As expected, non-transplanted control disease EPP mice showed markedly elevated levels of bilirubin and liver enzymes in the serum. Moreover, histologic examination of hematoxylin and eosin-stained liver sections taken from representative recipients of BALB/C or EPP donor marrow sacrificed 3 mo after transplantation showed no evidence of biliary cirrhosis, regenerative hyperplasia, or extracellular deposits of protoporphyrin in BALB/C recipients of either normal BALB/C or EPP donor marrow (Fig 3). In contrast, examination of liver sections of non-transplanted EPP control mice showed extensive areas of biliary cirrhosis, regenerative hyperplasia, and extracellular protoporphyrin deposits as previously described for this mouse model (Tutois *et al*, 1991). These results were consistent out to 16 mo after transplant, the longest time point tested. Together, these data demonstrate that the extremely high levels of blood protoporphyrin generated following transplantation of BALB/C mice with EPP bone marrow is insufficient to generate the severe liver damage observed in the EPP mouse model.

BALB/C recipients of EPP marrow show minimal skin photosensitivity despite high levels of plasma and erythrocyte protoporphyrin Skin photosensitivity, the clinical hallmark of EPP in humans, occurs as a result of excitation of the circulating protoporphyrin molecule by 400–410 nm light near the surface of the skin. The excited protoporphyrin reacts with molecular oxygen to produce a reactive oxygen species that causes damage to cell membranes and mitochondria. To investigate whether greatly elevated levels of plasma and erythrocyte protoporphyrin levels were sufficient to generate the photosensitive phenotype, transplanted and control mice were taken at 3 mo post-transplantation and shaved, depilated, and exposed to 20 min of a mercury vapor lamp that mimics exposure to

Table I. Assessment of liver function

Transplanted mice (number of mice)	Total bilirubin (mg per dL)	Direct bilirubin (mg per dL)	Indirect bilirubin (mg per dL)	Alkaline phosphatase (U per liter)	ALT (U per liter)	AST (U per liter)
Non-Tx BALB/C (2)	0.22 $\pm$ 0.01	0.01 $\pm$ 0	0.23 $\pm$ 0.01	111 $\pm$ 4.9	35 $\pm$ 4.2	49 $\pm$ 2.8
Non-Tx EPP (2)	0.6 $\pm$ 0.03	0.34 $\pm$ 0.04	0.25 $\pm$ 0.01	2109 $\pm$ 107	754 $\pm$ 47	838 $\pm$ 15
BALB/C Tx with BALB/C (3)[a]	0.3 $\pm$ 0.05	0.01 $\pm$ 0.01	0.29 $\pm$ 0.04	159 $\pm$ 33	41 $\pm$ 12.5	80 $\pm$ 24.8
BALB/C Tx with EPP (4)[a]	0.23 $\pm$ 0.03	0.01 $\pm$ 0.01	0.22 $\pm$ 0.03	141 $\pm$ 3.7	36 $\pm$ 1.9	53 $\pm$ 3.9

[a]One mouse from each group was sacrificed 3 mo after transplantation for liver histology and were not available for liver function analysis 12 mo after transplantation.
ALT, alanine amino-transferase; AST, aspartate amino-transaminase; Tx, transplanted; EPP, erythropoietic protoporphyria. Non-transplanted BALB/C and EPP mice were age matched with transplanted recipients.
Mice were analyzed 12 mo after transplantation. Data represent mean $\pm$ standard deviation.

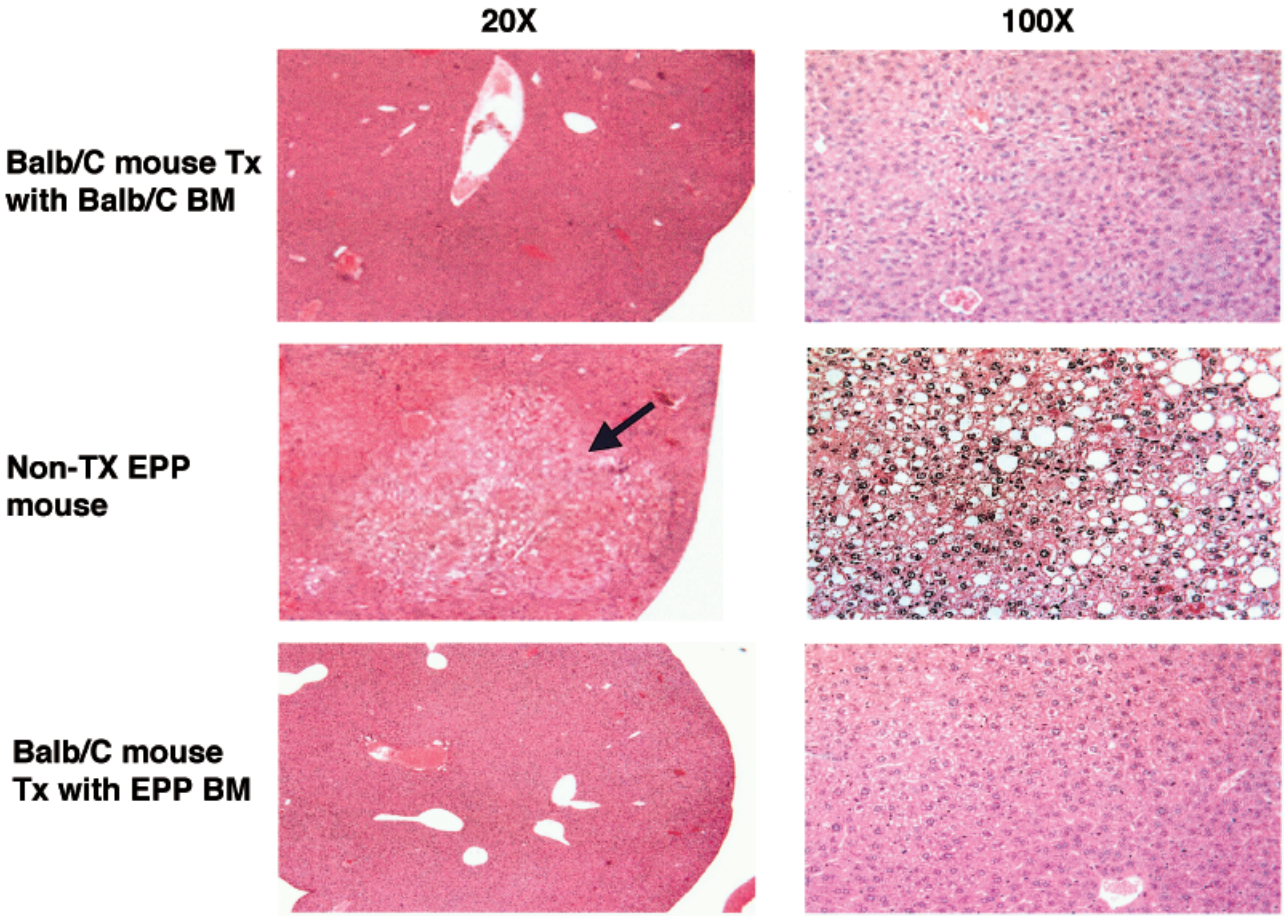

Figure 3
Lack of liver disease in BALB/C recipients of erythropoietic protoporphyria (EPP) marrow. One representative BALB/C recipient of BALB/C or EPP marrow was sacrificed 3 mo post-transplantation and the livers processed for histopathology. Sections were stained with hematoxylin/eosin and analyzed by a Board Certified Veterinary Pathologist. Sections analyzed at × 20 magnification (*left panels*) show the presence of large regenerative nodules (*arrow*) in the non-transplanted (Non-Tx) EPP mouse but not in livers of BALB/C recipients of either BALB/C or EPP marrow. Tissue sections analyzed at × 100 magnification (*right panels*) showed biliary cirrhosis, large vacuoles, and extracellular protoporphyrin deposits in the liver of the non-transplanted EPP mouse whereas no abnormalities were observed in the livers of BALB/C recipients of either BALB/CV or EPP marrow. BM, bone marrow.

sunlight. As expected, normal BALB/C mice showed no evidence of burns or lesions on their backs whereas non-transplanted control EPP disease mice showed severe photosensitivity (Fig 4*A* and *B*). Surprisingly, those BALB/C recipients of EPP donor marrow showed only minimal evidence of photosensitivity despite having extremely high levels of protoporphryin in their erythrocytes and plasma (Fig 4*C* and *D*). Similar results were observed out to 16 mo post-transplantation, the longest time point tested. These data suggest that the expression of normal levels of ferrochelatase in the skin is sufficient to provide a significant protection against photosensitivity despite the greatly elevated levels of photoreactive protoporphyrin in the plasma.

Expression of normal levels of ferrochelatase in skin protects against photosensitivity In order to conclusively demonstrate that normal levels of ferrochelatase enzyme produced by the skin can provide a significant photoprotective effect despite high levels of photoreactive protoporphyrin in the blood, we performed skin transplants between normal BALB/C mice and disease EPP mice. Circular skin grafts of approximately 1.0 cm in size were taken from the lumbar region of the dorsum of BALB/C (n = 2) and EPP disease (n = 2) mice with a scalpel blade, switched, and sutured into place. One mouse from each of the BALB/C and EPP background groups died approximately 3–4 d post-surgery presumably because of the procedure. The grafts on the surviving mice rapidly became vascularized and showed no signs of necrosis or tissue damage. One-week post-transplantation, the concentration of erythrocytic protoporphyrin in each remaining mouse was determined. As expected, the mouse of the normal BALB/C background showed a low concentration of protoporphyrin (2296 nmol per liter) whereas the mouse of the EPP disease background showed a greatly elevated protoporphyrin concentration (13,671 nmol per liter). Next, in order to determine conclusively that the ferrochelatase enzyme produced by cells of the normal BALB/C skin graft is sufficient to provide a photoprotective effect within the EPP background, the two mice were exposed to a mercury vapor lamp (5.52 J per cm^2 of 400–410 nm light) 1 mo post-transplantation. As shown in Fig 5*B*, although the EPP disease mouse showed evidence of severe photosensitivity on most of its exposed back, the region corresponding to the location of the normal

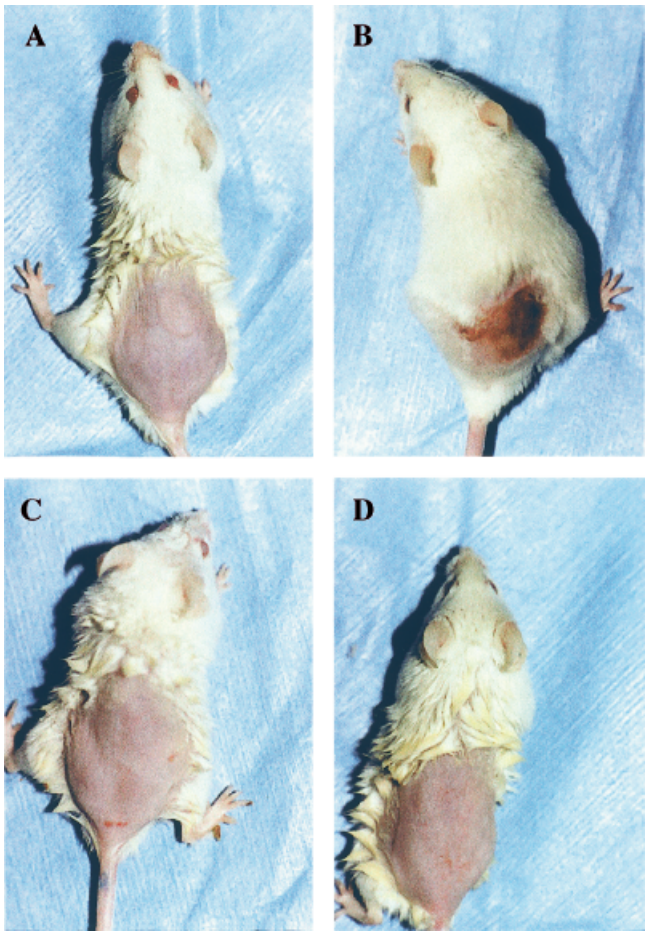

Figure 4
BALB/C recipients of erythropoietic protoporphyria (EPP) marrow show only very mild photosensitivity despite greatly elevated levels of circulating protoporphyrin. At 3 mo post-transplantation mice were shaved, depilated, and exposed to a mercury vapor lamp (5.52 J per cm^2 of 400–410 nm light) to mimic exposure to sunlight. Photographs were taken 5 d post-exposure. Non-transplanted (Non-Tx) normal BALB/C (*A*) and EPP (*B*) mice were used as controls. The bottom panels show two BALB/C recipients transplanted with EPP marrow (*C*, *D*). Note the very mild photosensitivity (small areas of mild ulceration) as compared with the non-transplanted EPP mouse (large areas of severe ulceration and crusting).

BALB/C skin graft showed only minimal evidence of photosensitivity. As expected, since the levels of protoporphyrin are not elevated in normal BALB/C mice, the BALB/C recipient of the EPP skin graft showed no evidence of photosensitivity either in the grafted EPP skin or in the surrounding normal skin.

Discussion

The main pathological feature of EPP, skin photosensitivity, which in some patients is severe, prevents them enjoying a normal lifestyle including outdoors activities. In some cases even exposure to artificial light causes symptoms (Kappas *et al*, 1995). Moreover, a certain percentage of patients also suffer from hepatic dysfunction, which in some cases leads to terminal liver failure (Bloomer *et al*, 1996). Previous experiments using the EPP mouse model has clearly shown that the major source of excess protoporphyrin derives from the erythroid compartment (Fontanellas *et al*, 2000) because of the extremely high level of porphyrin metabolism occurring in these cells. Our results confirm this previous observation. Transplant of bone marrow cells obtained from EPP disease donor mice into irradiated, normal BALB/C recipients resulted in a high level of reconstitution of the hematopoietic system with cells of the donor EPP phenotype leading to greatly elevated levels of erythrocyte and plasma protoporphyrin indistinguishable from control, non-transplanted EPP disease mice. Moreover, FACS analysis of red blood cells from BALB/C mice transplanted with EPP marrow showed that most cells produced an abnormal, protoporphyrin-specific fluorescent phenotype.

Interestingly, despite these greatly elevated levels of erythrocyte and plasma protoporphyrin, BALB/C recipients of EPP marrow showed no evidence of liver dysfunction as assessed by monitoring serum levels of bilirubin, alkaline phosphatase, and transaminases out to 16 mo post-transplantation. Moreover, histopathologic examination of liver sections from sacrificed animals showed no evidence of biliary cirrhosis, regenerative hyperplasia, extracellular deposition of protoporphyrin, abnormalities characteristically observed in 100% of non-transplanted EPP disease mice. Fontanellas *et al* (2000) has shown that transplantation of normal congenic mouse bone marrow into very young recipient EPP disease mice can prevent hepatobiliary complications and partially reverse protoporphyrin accumulation in the liver. Our results extend this earlier study by demonstrating that high levels of blood protoporphyrin is in itself insufficient to generate liver damage and that lack of ferrochelatase activity in cells of the liver plays a critical role in the development of the EPP-associated liver disease, at least in the mouse model. In humans the situation remains less clear. In a study of 112 EPP patients from 93 EPP families, Minder *et al* (2002) found a significant association between the possession of null-allele mutations that cause major structural alterations in the ferrochelatase enzyme, leading to the accumulation of especially high levels of protoporphyrin in the blood, and protoporphyrin-related liver disease in EPP. Moreover, in a follow-up study of seven EPP patients who received a liver transplantation, two patients had a recurrence of protoporphyric liver disease as assessed by liver chemistries and histopathological analysis of liver biopsies (Bloomer *et al*, 1996). The remaining five patients all showed normal liver chemistries and liver biopsies (Bloomer *et al*, 1996). It is important, however, to note that the two patients with recurrent liver disease following liver transplantation also showed signs of liver rejection, cytomegalovirus infection, and biliary tract obstruction that may have played a role in the development of damage to the transplanted liver (Bloomer *et al*, 1996). Conversely, Chen *et al* (2002) concluded from a study of 34 EPP patients from 24 EPP families that null mutations leading to high levels of blood protoporphyrin levels could not, by themselves, account for the severe liver phenotype since the same mutations were also observed in EPP patients not suffering from liver disease at the time of the study. A possible explanation for these discordant observations is that the patients with null mutations and greatly elevated levels of blood protoporphyrin but no clinically detectable liver disease were on their way to developing liver disease, but

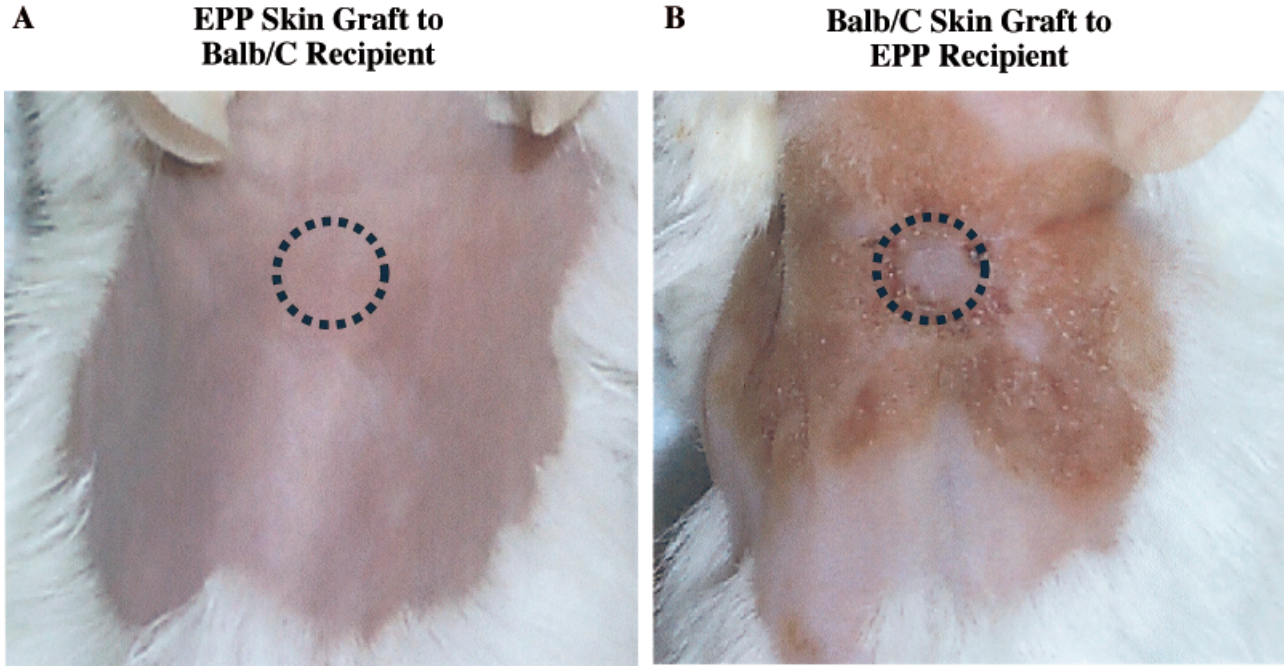

Figure 5
Normal levels of ferrochelatase expressed in the skin protects against photosensitivity. One month following skin transplantation the backs of mice were shaved, depilated, and exposed to 20 min of a mercury vapor lamp. Areas of the skin graft are circled. The erythropoietic protoporphyria (EPP) recipient of the BALB/C skin graft shows burns over a large area of the back. Note the lack of burns within the area of the normal skin graft.

insufficient time had passed for the disease to become detectable. Alternatively, other genetic and/or acquired factors may play a critical role in the development of EPP-associated liver disease.

The most critical pathological feature of both murine and human EPP is skin photosensitivity. We and others have previously demonstrated that transplantation of either normal murine HSC or murine EPP HSC transduced with a retroviral vector encoding the wild-type ferrochelatase cDNA into EPP disease recipient mice can cure the skin photosensitivity observed in murine EPP (Pawliuk *et al*, 1999; Fontanellas *et al*, 2000, 2001; Richard *et al*, 2001). In all of these studies, the transplantation of wild-type or genetically corrected EPP HSC resulted in a normalization of blood protoporphyrin levels leading to long-term cure of the photosensitive phenotype. Interestingly, in this study we found that despite massive elevation in blood protoporphyrin levels in BALB/C recipients of EPP donor marrow, photosensitivity was almost completely abrogated. These results suggest that the expression of normal levels of ferrochelatase enzyme in the skin is sufficient to metabolize the local circulating levels of protoporphyrin and prevent photosensitivity. This conclusion is further supported by the observation of a photoprotective effect within a region of normal skin following transplant onto the back of an EPP disease mouse. This observation raises the interesting possibility that as an alternative to HSC gene therapy, more localized treatment of exposed regions of skin with a therapeutic onco-retroviral or lentiviral vector might provide a sufficient corrective effect to enable patients to engage in outdoor activities. We are currently investigating the photoprotective effect following lentiviral-mediated transfer of a functional ferrochelatase cDNA to the dermal cells of EPP mice. This localized treatment, however, would not have an effect upon the high circulating levels of protoporphyrin within the blood and would not, therefore, prevent the development of liver damage.

This study has confirmed that the bone marrow is the main source of excess protoporphyrin and has demonstrated that high circulating levels of protoporphyrin within the blood is insufficient by itself to lead to the development of the liver damage and the photosensitivity characteristic of murine EPP. The development of methods to stably and efficiently deliver a functional ferrochelatase enzyme locally into the dermis of EPP mice should contribute to the development of successful clinical protocols for the treatment of human EPP.

Materials and Methods

Mice BALB/C-Fech^{m1Pas} (hereafter referred to as EPP mice) and BALB/C mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). Eight- to 12-wk-old female and male mice were used, respectively, as donors and recipients. All experiments involving animals were approved by the Massachusetts Institute of Technology Committee on Animal Care.

Bone marrow transplantation Femoral bone marrow cells were harvested from female EPP or BALB/C mice following sacrifice. Cells were spun down, suspended in Hanks balanced salt solution (Invitrogen, Carlsbad, California) containing 2% fetal bovine serum (Hyclone, South Logan, Utah), counted and injected intravenously into each male BALB/C mouse given 880 cGy of whole-body irradiation (80 cGy per min ^{137}Cs γ-rays).

Erythrocyte protoporphyrin concentrations and skin photosensitivity assay Erythrocyte protoporphyrin was measured as previously described (Piomelli, 1977). The backs of mice were shaved and depilated. An hour after depilation, the mice were

exposed to a mercury vapor lamp (Spectroline, model BIB-150B, Spectronics Corp., Westbury, New York) that was filtered with window glass to remove ultraviolet B radiation. The spectral output of the filtered lamp was 320–580 nm and the dose of 400–410 nm radiation was 5.52 J per cm^2. The mice were observed daily for the development of skin lesions and photographed at 5 d post-irradiation. Control experiments demonstrated that depilated non-porphyric BALB/C mice could be exposed to 1 h of mercury vapor lamp radiation and still not develop any skin lesions.

Histopathology Livers were removed from mice following sacrifice and placed in 10% neutral buffered formalin (VWR, Batavia, Illinois). Sections were prepared and stained with hematoxylin and eosin. Analysis of sections was performed by a trained Veterinary Pathologist.

Skin transplants Normal, BALB/C and diseased EPP mice were anesthetized with 300 μL of 2.5% of freshly prepared Avertin. The backs of the mice were shaved, depilated, and wiped with disinfectant. A circular patch of skin approximately 1.0 cm in diameter was cut from the lumbar region of the dorsum of both BALB/C and EPP disease mice with a scalpel blade. Next, the grafts were switched so that they were placed onto the backs of recipient mice of the opposite phenotype and the grafts were sutured into place with 5-0 silk. Mice were bandaged, wrapped with cloth tape, and monitored until fully recovered. Upon recovery, erythrocyte protoporphyrin concentrations and skin photosensitivity was assessed as described above.

Statistical analysis The Student *t* test was utilized to determine statistical significance. p-values are indicated in the Results.

We are grateful to James Murphy from the MIT Division of Comparative Medicine for expert pathology. This work was supported by NIH grant 5RO1DK056174 to M. M.-R.

DOI: 10.1111/j.0022-202X.2004.23529.x

Manuscript received June 2, 2004; revised August 11, 2004; accepted for publication August 13, 2004

Address correspondence to: Dr Philippe Leboulch, Harvard New Research Building, Genetics Division (4th floor), 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA; Dr. Micheline M. Mathews-Roth Channing Laboratory, Harvard Medical School, 181 Longwood Avenue, Boston, Massachusetts 02115, USA. Emails: paulvw@mit.edu, mmmathroth@rics.bwh.harvard.edu

References

Bloomer JR: The porphyrins. In: Schiff ER, Sorrel MF, Maddrey WC (eds). Schiff's Diseases of the Liver, 8th edn. Philadelphia: Lippincott-Raven Publications, 1999; p 1151–1178

Bloomer JR, Rank JM, Payne WD, Snover DC, Sharp HL, Zwiener RJ, Carithers RL: Follow-up after liver transplantation for protoporphyric liver disease. Liver Transplant Surg 2:269–275, 1996

Boulechfar S, Lamoril J, Montagutelli X, *et al*: Ferrochelatase structural mutant ($Fech^{m1Pas}$) in the house mouse. Genomics 16:645–648, 1993

Chen FP, Risheg H, Liu Y, Bloomer J: Ferrochelatase gene mutations in erythropoietic protoporphyria: Focus on liver disease. Cell Mol Biol 48:83–89, 2002

Desnick R: In: Isselbacher KJ, *et al*: (eds). The prophyrias. In: Harrison's Principles of Internal Medicine, Vol. 1, 13th edn. New York: McGraw-Hill, 1994: 2073

Fontanellas A, Mazurier F, Landry M, *et al*: Reversion of hepatobiliary alterations by bone marrow transplantation in a murine model of erythropoietic protoporphyria. Hepatology 32:73–81, 2000

Fontanellas A, Mendez M, Mazurier F, *et al*: Successful therapeutic effect in a mouse model of erythropoietic protoporphyria by partial genetic correction and fluorescence-based selection of hematopoietic cells. Gene Ther 8:618–626, 2001

Kappas A, Sassa S, Galbraith RA, Nordmann Y: The porphyrias. In: Schriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease, 7th edn. New York: McGraw-Hill, 1995; p 2103–2159

Key NS, Rank JM, Freese D, Bloomer JR, Hammerschmidt DE: Hemolytic anemia in protoporphyria: Possible precipitating role of liver failure and photic stress. Am J Hematol 39:202–207, 1992

Mathews-Roth MM, Pathak MA, Fitzpatrick TB, Harber LH, Kass EH: Beta carotene therapy for erythropoietic protoporphyria and other photosensitivity diseases. Arch Dermatol 113:1229–1232, 1977

Minder EI, Gouya L, Schneider-Yin X, Deybach JC: A genotype–phenotype correlation between null-allele mutations in the ferrochelatase gene and liver complication in patients with erythropoietic protoporphyria. Cell Mol Biol 48, 91–96, 2002

Pawliuk R, Bachelot T, Wise RJ, Mathews-Roth MM, Lebouldh P: Long-term cure of the photosensitivity of murine erythropoietic protoporphyria by preselective gene therapy. Nat Med 5:768–773, 1999

Piomelli S: Free erythrocyte porphyrins in the detection of undue absorption of Pb and of Fe deficiency. Clin Chem 23:264–269, 1977

Poh-Fitzpatrick MB, Wang X, Anderson KE, Bloomer JR, Bolwell B, Lichtin AE: Erythropoietic protoporphyria: Altered phenotype after bone marrow transplantation for myelogenous leukemia in a patient heteroallelic for ferrochelatase gene mutations. J Am Acad Dermatol 46:861–866, 2002

Richard E, Mendez M, Mazurier F, *et al*: Gene therapy of a mouse model of protoporphyria with a self-inactivating erythroid-specific lentiviral vector without preselection. Mol Ther 4:331–338, 2001

Tutois S, Montagutelli X, Da Silva V, *et al*: Erythropoietic protoporphyria in the house mouse. A recessive inherited ferrochelatase deficiency with anemia, photosensitivity, and liver disease. J Clin Invest 88:1730–1736, 1991